THE HYMENOPTEROUS POISON APPARATUS.
VI. *CAMPONOTUS PENNSYLVANICUS* (HYMENOPTERA: FORMICIDAE)

By Henry R. Hermann and Murray S. Blum
University of Georgia, Athens

METHODS AND MATERIALS

Workers of *Camponotus pennsylvanicus* were collected in Baton Rouge, La., in 1966 and 1967. Glandular and reservoir regions were examined for morphological details after dissection of live specimens in normal saline. Sclerites were removed from the abdomen, dehydrated in ethyl alcohol, cleared in xylene and mounted in Permount. Some apparatuses were examined whole while the individual sclerites of others were disarticulated and examined individually.

For preparations of histological sections, glands were removed in normal saline, dehydrated in ethyl alcohol, cleared in xylene and embedded in Paraplast. Tissue was sectioned at 10 microns, stained with Delafield's hematoxylin and eosin Y, and mounted on slides with Permount. All measurements for illustrations are in millimeters.

In preparation for chemical analysis, poison sacs were dissected from workers which had been relatively immobilized by storing them at 4°C for several hours. The glands were rinsed in distilled water and the venom was transferred to filter paper by puncturing the reservoir with a fine needle.

The venom-impregnated filter papers were subsequently treated with several diagnostic organic agents including Fehling's reagent, 2, 4-dinitrophenylhydrazine and ninhydrin. Ultimately, a large series of compounds present in the poison gland secretion were resolved by applying the contents of fifty glands to the origin of 14½" × 13" sheets of Whatman #1 filter papers. The venomous constituents were analyzed by two-dimensional chromatography by employing *n*-butanol: acetic acid: water (4:1:1) as the first phase (17 hr) (Reed, 1950) and 80% aqueous pyridine as the second (8 hr) (Flavin and Anfinsen, 1954). The compounds were detected by spraying the papers with 0.2% ninhydrin in acetone following which the chromatograms were heated at 100°C for 10 minutes. Standard mixtures of amino acids were run as controls in the same cabinet as the venom-treated paper and in some cases amino acid mixtures were co-chromatographed with the poison gland contents.

Much of the literature concerning the hymenopterous poison apparatus has been cited by Hermann and Blum (1966, 1967a, 1967b). One of the first comparative investigations on the hymenopterous poison apparatus that included descriptions on the apparatus of formicine ants was undertaken by Forel (1878). Since that time, Foerster (1912) contributed considerably to an understanding of the formicine poison apparatus, his work involving the skeletal and muscular components to describe a functional system.

Emery (1922) and Buren (1944) noted that the nozzle-like projection at the tip of the gaster of formicine ants is distinct from the cloacal orifice. As reviewed by Brown (1954), this projection forms a cone by an inrolling of the posterior portion of sternum VII and functions as a channel through which venom is sprayed to a considerable distance. This cone often has been misidentified as the cloacal orifice.

Hung and Brown (1966) dealt in detail with this nozzle-like structure in the Formicinae, calling it the acidopore. It was pointed out that the ring of fine setae surrounding the acidopore, the coronula, directs the venom spray outward away from the ant's body. The acidopore is situated on the ventral apex of sternum VII. In certain species of the Camponotini, the acidopore may be formed as much by tergum VII as by sternum VII.

Carthy (1951), Wilson (1963) and Blum and Wilson (1964) reported that the odor trail pheromone in certain formicine species was a product of the hindgut or some structure associated with the hindgut. Knowledge of this source of pheromone distinguishes this subfamily from some other subfamilies of ants in which the trail pheromone is a product of the poison gland (Blum and Moser, 1963; Blum et al., 1964), Dufour's gland (Wilson, 1959, 1962) or Pavan's gland (Wilson and Pavan, 1959).

Further anatomical investigations of the formicine poison apparatus were reported by Maschwitz (1964). In his work, Maschwitz described the poison sac and associated structures, Dufour's gland and the method of venom ejection in certain formicine species.

We undertook the present investigation in part to describe the soft and sclerotized regions that make up the poison apparatus in *Camponotus pennsylvanicus* (DeGeer). In an effort to characterize chemically the venoms of higher formicine ants, we undertook to identify the minor constituents that accompany formic acid in the venom of this species. At this juncture, we concentrated our efforts on establishing the chemical nature of the compounds secreted by the true poison gland in contradistinction to any components that may

be elaborated by Dufour's gland and ultimately mixed with the poison gland secretion. The venom produced by the poison glands of formicine ants has long been identified with formic acid, and it is quite clear that this compound is a consistent chemical denominator for the venomous secretions produced by members of the Formicinae.

However, arthropod secretions usually have many components, and formicine venoms do not appear to violate this generalization. Thus, Stumper (1959) has demonstrated that the venom of *Formica polyctena* Förster contains in addition to formic acid, at least two other minor constituents. Similarly, Ghent (1961) reported that the venom of *Camponotus pennsylvanicus* (DeGeer) was characterized by the presence of a water-soluble solid which constituted about 5% of the venom.

This research is part of a comparative study of the hymenopterous poison apparatus. An investigation of other formicine species is presently underway and will be reported on in forthcoming publications.

RESULTS

The poison sac (PS) differs from the sacs of ants in other subfamilies in that there is no gland invaginated into the sac (Fig. 2, A, B). Instead, an extremely long and narrow convoluted duct (CG) lies adnate to the dorsal surface of the sac. This duct branches into two long free filaments (FF) at the base of the sac. These filaments maintain a relatively uniform diameter throughout their length.

The basic composition of the reservoir, convoluted duct and free filaments is the same in this species as in other formicids previously described (Forel 1878, Maschwitz 1964). The difference lies in the position of the convoluted region. In most formicids, the convoluted portion, responsible for enzymatic activity in changing precursory compounds picked up by the free filaments, is totally within the sac (unpublished data). In *C. pennsylvanicus,* this region lies on the outside of the sac, and by pulling on the base of the free filaments, the elongate convoluted duct can be unraveled.

Fig. 1. Comparison between the poison apparatus of *Camponotus pennsylvanicus* and what may be considered a typical stinging ant. A-Apparatus similar to that found in ponerine ants (LV). B-Transverse section through sting of a stinging ant species. C-Distal tip of sting showing barbed lancet tips extending posteriad from tip of sting shaft (LV). D-Poison apparatus of *C. pennsylvanicus* (LV).
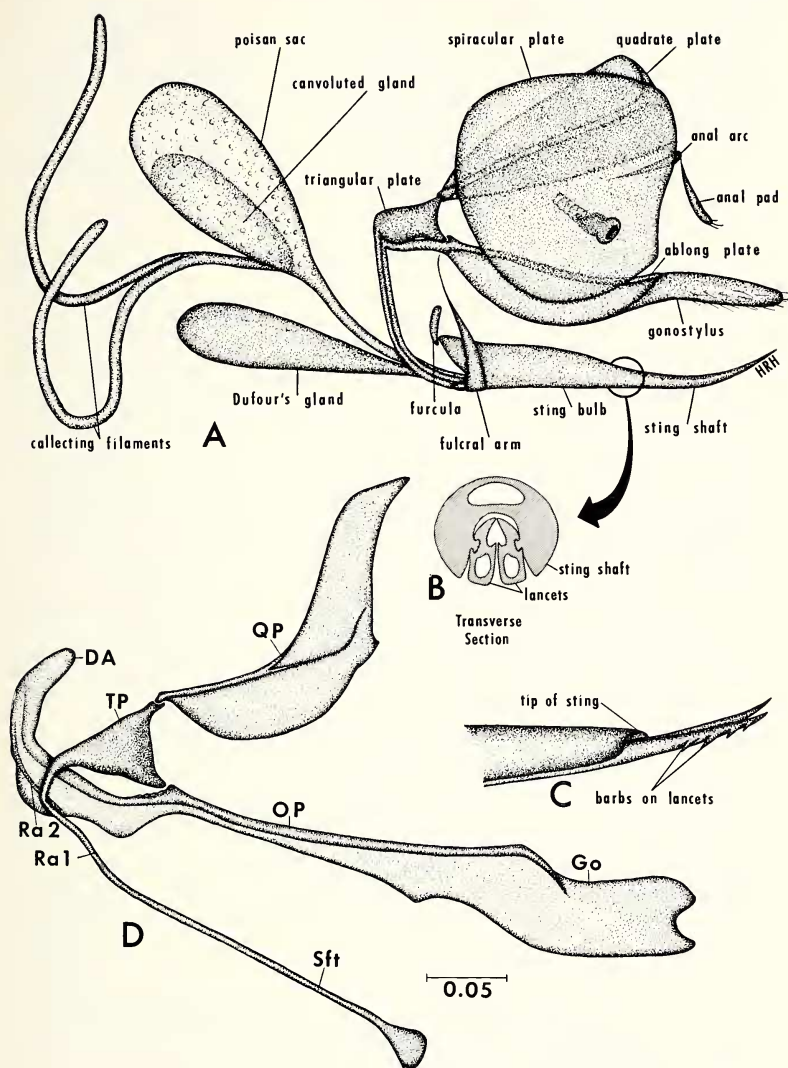
FIGURE 1

The sac is surrounded by a simple muscular layer which functions in forcing venom out of the sac. The convoluted region receives an abundant tracheal supply.

Dufour's gland (DG) in *C. pennsylvanicus* differs markedly from those glands in other subfamilies of ants. Instead of being an elongate unilobular sac, as is commonly found in ants previously examined, Dufour's gland in this species is bilobed (Fig. 2, C). A bilobed Dufour's gland has been reported in *Formica rufibarbis* by Forel (1878) and in *Formica polyctena* by Maschwitz (1964).

The components that make up the sclerotized portion of the poison apparatus (Fig. 1, D) are basically similar to those in stinging Hymenoptera (Fig. 1, A, B, C). However, some of the major sclerites have become reduced almost to a point beyond recognition.

The oblong plate is relatively well developed (OP, Fig. 1, D; Fig. 2, H). However, its ramus (Ra 2) has been reduced considerably, so that now it is represented by a thin and slightly sclerotized bar only near the proximal end of the oblong plate. The fused second valvulae (sting) are wanting. Consequently, the levator muscle of the sting, normally originating on the posterior border of the second ramus and inserting on the anterior region of the sting bulb (SB, Fig. 2, G), is also wanting.

Although gonostyli are present (Go, Fig. 1, D; Fig. 2, I), they are membranous and possess minute setae (Set) along the lateral and ventrolateral regions. Most stinging Hymenoptera possess long setae on each gonostylus (Fig. 1, A), especially in the caudal and ventro-caudal region.

The first valvifers (triangular plates, TP, Fig. 1, D; Fig. 2, E) are triangular in appearance, and each articulates anteriorly to a slender ramus (Ra 1). In stinging Hymenoptera, each of the rami articulates ventrally with an elongate lancet shaft (LS) the latter usually terminating distally as a pointed and barbed structure (Fig. 1, C). However, in *C. pennsylvanicus* each first valvifer is no longer a lancet shaft, but an elongate rod that terminates in a spatulate distal end (Fig. 1, D; Fig. 2, E). The valve, a structure that

Fig. 2. Components of the poison apparatus of *C. pennsylvanicus* and a stinging ant species (LV). A-Transverse section of poison sac. B-Poison sac of *C. pennsylvanicus* (DV). C-Dufour's gland of *C. pennsylvanicus* (DV). D-Lancet of *Paraponera clavata* (LV). E-Lancet of *C. pennsylvanicus* (LV). F-Spiracular plate of *C. pennsylvanicus* (LV). G-Oblong plate, second ramus, gonostylus, fulcral arm and sting of *P. clavata* (LV). H-Oblong plate, gonostylus and portion of second ramus of *C. pennsylvanicus* (LV). I-Gonostylus of *C. pennsylvanicus* (LV).
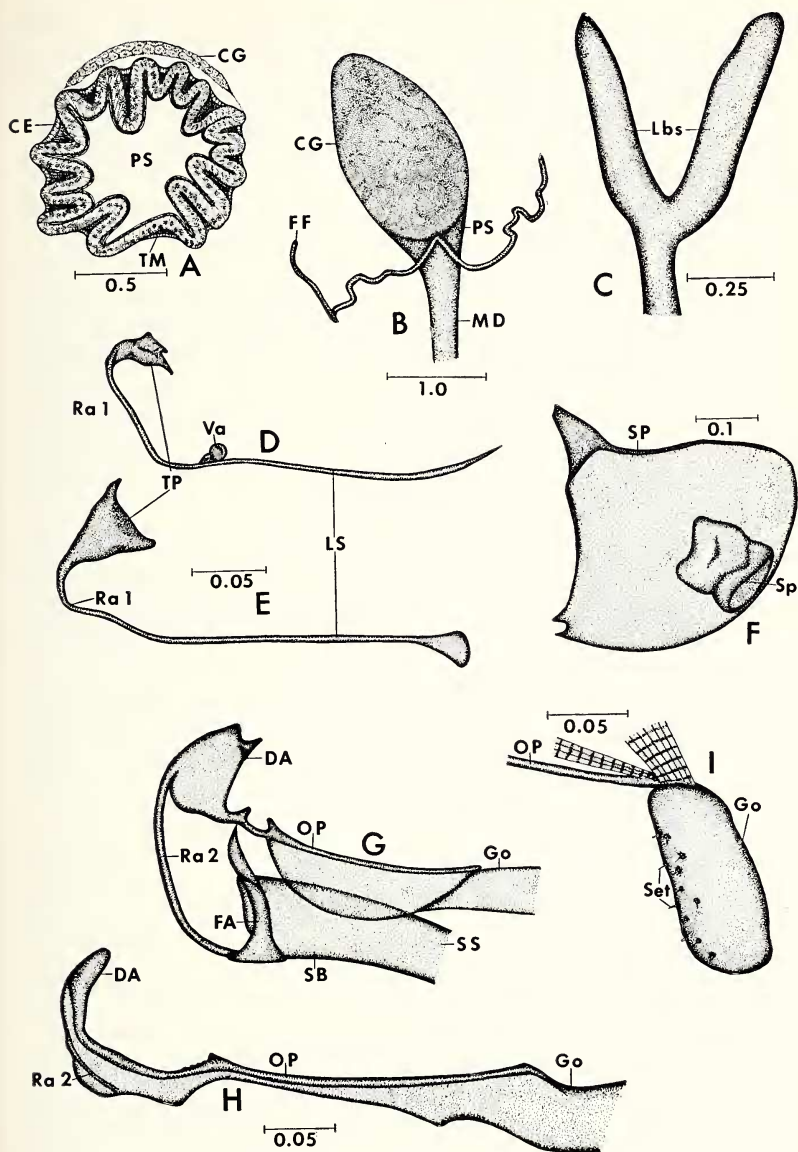
FIGURE 2

functions in forcing venom through the sting shaft in stinging species (Va, Fig. 2, D), is lacking in *C. pennsylvanicus*.

Each of the two quadrate plates (QP, Fig. 1, D) is anatomically similar to those found in most stinging Hymenoptera (Fig. 1, A). The distal region of each quadrate plate acts as a point of insertion for a muscle originating on the anterior and posterior regions of the oblong plate. The quadrate plate articulates anteroventrally with the dorsal apodeme of the triangular plate (TP).

Neither fulcral (FA, Fig. 1, A) arms nor a furcula (Fu) were found in this species. Both the fulcral arms and furcula articulate with the anterior end and anteroventral region of the sting bulb (SB, Fig. 1, A) in stinging species. Since the sting bulb is wanting, at least 4 muscle groups that normally insert or have their origin on it are also wanting. In stinging forms, these muscles typically serve to deflect and rotate the sting, and pass over the poison canal to function as a sphincter in closing the passage through which venom issues during the act of stinging.

The spiracular plates (8th hemitergites, SP, Fig. 2, F) are basically the same as those in other hymenopterans (Fig. 1, A). Since the general shape of these structures changes considerably throughout the Hymenoptera, it is difficult to discuss any significant differences between this and other species at this point.

Aside from formic acid, which constitutes nearly 50% of the volume of the poison gland secretion (Ghent 1961), the venom contains one other obvious constituent, a non-volatile residue. The existence of this water-soluble powder was noted by Ghent (1961), who estimated that it represented about 5% of the whole venom.

The residue does not have any pronounced odor and is relatively insoluble in organic solvents, especially those that are non-polar. A clue to the identities of at least some of the components in the residue was obtained after it was observed that the powdery deposit reacted intensely with ninhydrin. After analysis by two-dimensional chromatography, it became evident that the venom of *C. pennsylvanicus* contains a large series of free amino acids.   .

Thirteen amino acids were detected in the poison gland secretion. Based on the intensities of the colored ninhydrin-complexes, leucine, valine and serine appeared to be present in the highest concentrations. Lysine, proline, alanine, glutamine and χ-aminobutyric acid were present in lower concentrations. Cystine, glycine, arginine, aspartic acid and threonine were minor constituents. A small amount of ninhydrin-positive material remained at the origin.

DISCUSSION

Based on this investigation and descriptions of the poison apparatus of other formicine species, we can describe an apparatus that may be considered typical for the subfamily Formicinae. Without exception, the poison apparatus of formicine ants, including previously investigated species and several other species in our investigations not discussed here, (1) is basically similar in appearance to that of stinging species, although (2) the gonapophyses that form the 2nd valvulae (sting bulb and shaft) are wanting; (3) there is no valve on the lancets; (4) the tongue-and-groove articulations between lancets and sting shaft have been lost; (5) the fulcral arms are wanting; and (6) the gonostyli have been reduced to membranous structures.

This apparatus of *C. pennsylvanicus* is typical of formicines in other respects. The poison sac in all formicines investigated was large and possessed a convoluted structure on much of its dorsal surface. The free filaments extend from the base of the sac at the proximal end of the convoluted gland. Whether the poison sac is similar in *all* formicine species will have to be investigated, although this form holds true for at least two species of *Lasius* and a species of *Acanthomyops* as well as several species of *Camponotus* and *Formica*.

Dufour's gland in *C. pennsylvanicus* is typical for species in the genera *Camponotus* and *Formica* that we have examined, but not for some of the more primitive genera. In some species of *Lasius* and *Acanthomyops,* Dufour's gland is distinctly unilobular.

The presence of a large series of free amino acids in the venom of *C. pennsylvanicus* demonstrates for the first time, that formicine venoms share some common chemical characteristics with those of stinging ants. The majority of these amino acids are found in the poison gland secretion of the myrmicine *Tetramorium guineense* (F.) (Blum and Ross 1965), and free amino acids are also characteristic of other myrmicine venoms (Blum 1966). Thus, although the venoms of no non-formicine ants are known to contain formic acid, the assumption that the venoms of formicine species share no common chemical components with those of stinging ants is no longer tenable.

The venom of *C. pennsylvanicus* may be typical of formicines in being fortified with free amino acids. We have examined also the venom of *Formica pallidefulva* Latreille and detected the presence of free amino acids. It is not improbable that the water-soluble residue

that Stumper (1959) noted in the venom of a member of the *Formica rufa* complex is similarly composed of free amino acids.

It seems appropriate to ask what role, if any, free amino acids may play in enhancing the toxicity of the highly concentrated formic acid in the venom. Ghent (1961) has established that the formic acid in venom is spread over twice as large an area of the insect cuticle as the same concentration of control aqueous formic acid. He thus concludes that the white residue (amino acids) contributes to the toxicity of the formic acid by distributing the toxicant over a larger area than would be treated otherwise. However, it is worth bearing in mind that Stumper (1959) detected also an odorous constituent in the venom of a species of *Formica*. Stumper speculates that this volatile component may have arisen from the Dufour's gland secretion, thus introducing the possibility that the secreted venom of formicines may contain products originating in two glands. In view of this distinct possibility, it is premature to attempt to explain the roles of poison gland products without considering the probably significant contribution to the toxicity of formicine venoms that the Dufour's gland products may make.

It should by no means be concluded that the chemistry of the formicine poison gland secretion is elucidated completely. The poison gland contents of *C. pennsylvanicus* contain, in addition to the described compounds, three compounds that reduce aromatic amino salts after the poison gland secretion has been subjected to thin layer chromatographic analysis. These compounds do not correspond to any amino acids, and they must represent unidentified constituents characteristic of the venom of this species. It may be no exaggeration to state that the elaborate formicine poison gland may yet be demonstrated to be a rich source of unsuspected natural products.

SUMMARY

Although *Camponotus pennsylvanicus* has well defined defensive mechanisims of biting and introducing acid into the wound, or merely the spraying of acid and other substances, some of the sclerites that take part in stinging in more primitive formicids are markedly reduced in this species. However, the glands and reservoir regions associated with the apparatus are well developed.

The white residue in the dry poison gland secretion consists of a series of 13 amino acids. Leucine, valine and serine are the major free amino acids present. The chemistry of formicine venoms and the possible roles played by their constituents are discussed.

LITERATURE CITED

BLUM, M. S.
  1966. The source and specificity of trail pheromones in *Termitopone,*
     Monomorium and *Huberia,* and their relation to those of some
     other ants. *Proc. Roy. Ent. Soc. Lond.* (A) 41: 155-160.
BLUM, M. S., J. C. MOSER AND A. D. CORDERO
  1964. Chemical releasers of social behavior. II. Source and specificity
     of the odor trail substances in four attine genera. (Hymenoptera:
     Formicidae). Psyche, 71: 1-7.
BLUM, M. S. AND G. N. ROSS
  1965. Chemical releasers of social behavior. V. Source, specificity and
     properties of the odor trail pheromone of *Tetramorium guineense*
     (F.) (Formicidae: Myrmicinae). *J. Insect Physiol.* 11: 857-868.
BLUM, M. S. AND E. O. WILSON
  1964. The anatomical source of trail substances in formicine ants.
     Psyche 71: 28-31.
BROWN, W. L., Jr.
  1956. Remarks on the internal phylogeny and subfamily classification
     of the family Formicidae, *Ins. Sociaux* 1: 21-31.
BUREN, W. F.
  1944. A list of Iowa ants. Iowa State Coll. Jour Sci. 18: 277-312.
CARTHY, J. D.
  1951. The orientation of two allied species of British ants. II. Odor
     trail laying and following in *Acanthomyops (Lasius) fuliginosus.*
     Behavior 3: 304-18.
EMERY, C.
  1922. L'ouverture clocale des Formicinae ouvrieres et femelles. Bull.
     Soc. Ent. Belg. 4: 62-5.
FLAVIN, M. AND C. B. ANFINSEN
  1954. The isolation and characterization of cysteic acid peptides in
     studies on ovalbumin synthesis. J. Biol. Chem. 211: 375-390.
FOERSTER, E.
  1912. Vergleichendanatomische Untersuchunge uber den Stechapparat
     der Ameisen. Zool. Jahrb. (Sect. 2). Abt. Anat. Ontog. Tiere.
     34: 347-80.
FORBES, J.
  1954. The anatomy and histology of the male reproductive system of
     Camponotus pennsylvanicus DeGreer (Formicidae, Hymenoptera)
     J. Morph. 95: 523-47.
FOREL, A.
  1887. Der Giftapparat und die Analdrusen der Ameisen. Zeitschr. f.
     wiss. Zool. 30: 28-66.
GHENT, R. L.
  1961. Adaptive refinements in the chemical defense mechanisms of cer-
     tain Formicinae. Ph.D. thesis. Cornell University (unpublished).
HERMANN, H. R., JR., AND M. S. BLUM
  1966. The morphology and histology of the hymenopterous poison ap-
     paratus. I. *Paraponera clavata* (Formicidae). Ann. Entomol.
     Soc. Amer. 59: 397-409.

1967a. The morphology and histology of the hymenopterous poison apparatus. II. *Pogonomyrmex badius* (Formicidae) Ann. Entomol. Soc. Amer. **60**: 661-8.

1967b. The morphology and histology of the hymenopterous poison apparatus. III. *Eciton hamatum* (Formicidae). Ann. Entomol. Soc. Amer. **60**: 1282-91.

HUNG, A. C. F., AND W. L. BROWN
1964. Structure of gastric apex as a subfamily character of the Formicinae (Hymenoptera: Formicidae). N. Y. Entomol. Soc. **74**: 198-200.

MASCHWITZ, U.
1964. Gefahrenatarmstoffe and Gefahrenalarmierung bei sozialen Hymenopteran. vergl. Physiol. **47**: 496-655.

MOSER, J. C. AND M. S. BLUM
1963. Trail marking substance of the Texas leaf-cutting ant: Source and Potency. Science **140**:1228.

REED, L. J.
1950. The occurrence of χ-aminobutyric acid in yeast extract: its isolation and identification. J. Biol. Chem. **183**: 451-458.

STUMPER, R.
1959. Un noveau constituent odorant du venin acide de fourmis. Compt. Rend. **249**: 1154-1156.

WILSON, E. O.
1959. Source and possible nature of the odor trail of the fire ant *Solenopsis saevissima* (Fr. Smith). Science **129**: 643-44.

1962. Chemical communication amoung workers of the fire ant *Solenopsis saevissima* (Fr. Smith). 1. The organization of mass-foraging. 2. An information and analysis of the odour trail. 3. The experimental induction of social responses. Animal Behavior. **10**: 134-64.

1963. The social biology of ants. Ann. Rev. Entomol. **8**: 345-68.

WILSON, E. O., AND M. PAVAN
1959. Source and specificity of chemical releasers of social behavior in the dolichoderine ants. Psyche **66**: 70-6.

ABBREVIATIONS USED IN FIGURES

CE	Columnar epithelium
CG	Convoluted gland
DA	Dorsal apodeme of oblong plate
DG	Dufour's gland
DV	Dorsal view
FA	Fulcral arms
FF	Free filaments
Fu	Furcula
Go	Gonostylus
Lbs	Lobes of Dufour's glands
LS	Lancet shaft
LV	Lateral view
MD	Main duct of poison sac
OP	Oblong plate
PS	Poison sac
QP	Quadrate plate
Ra 1	Ramus of first valvifer (triangular plate)
Ra 2	Ramus of second valvifer (oblong plate)
SB	Sting bulb
Set	Setae on gonostylus
Sft	Shaft of lancet
Sp	Spiracle
SP	Spiracular plate
SS	Sting shaft
TM	Transverse plate
TP	Triangular plate
Va	Valve of lancet